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Analysis of the Tyrosine Hydroxylase and Dopamine D4 Receptor Genes in a Croatian Sample of Bipolar I and Unipolar Patients

L. Oruč,[1] G.R. Verheyen,[1] I. Furač,[1,2] M. Jakovljević,[3] S. Ivezić,[4] P. Raeymaekers,[1] and C. Van Broeckhoven[1]*

[1]*Neurogenetics Laboratory, Flanders Interuniversity Institute for Biotechnology, Born-Bunge Foundation, University of Antwerp, Department of Biochemistry, Antwerp, Belgium*

[2]*Neurochemical Laboratory, Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Zagreb, Croatia*

[3]*Department of Psychiatry, University Hospital "Rebro," University of Zagreb, Zagreb, Croatia*

[4]*Psychiatric Hospital "Vrapće," School of Medicine, University of Zagreb, Zagreb, Croatia*

We selected 83 patients with bipolar disorder type I or unipolar recurrent major depression and 71 healthy controls for genetic analysis of the tyrosine hydroxylase and the dopamine D4 receptor gene. No significant association was found between bipolar disorder type I and unipolar recurrent major depression and the polymorphisms located near these genes. Therefore, the hypothesis that the tyrosine hydroxylase and the dopamine D4 receptor genes may be involved in the etiology of bipolar disorder and unipolar recurrent major depression is not supported in our study. Am. J. Med. Genet. 74:176–178, 1997.

KEY WORDS: genetics; association; mood disorders; tyrosine hydroxylase gene; dopamine D4 receptor gene

INTRODUCTION

Although the etiology of mood disorders is unknown, it appears that genetic factors may contribute to the development of both bipolar (BP) and unipolar (UP) disorder [McGuffin et al., 1994]. If they are due to a complex or multifactorial inheritance, it may be feasible to identify the genetic components using a case-control association strategy with candidate genes [Hodge, 1993].

Variations in dopamine receptor genes could be implicated in the pathogenesis of BP and UP disorders [Mitchell et al., 1992]. The dopamine D4 receptor gene (DRD4) has a highly polymorphic 48-bp Variable Number of Tandem Repeats (VNTR) polymorphism in exon III. The varying number of repeated segments reflects the differences in affinity of the receptor to bind clozapine in vitro [Van Tol et al., 1991, 1992]. These variations therefore might affect the functional efficiency of the D4 receptor, making this gene a noteworthy candidate for assessing dopamine-related behavior.

Since tyrosine hydroxylase (TH) is the rate-limiting enzyme for the biosynthesis of dopamine, genetic variants of the TH gene might play a role in BP and UP disorders. As the TH and DRD4 genes are genetically and physically close [Lim et al., 1993], and since both genes encode for proteins involved in dopaminergic neurotransmission, there may be an interactive regulation between TH and DRD4 at the DNA level [Sidenberg et al., 1994]. Thus, it is of value to perform genetic studies in this region and to explore the role of dopamine-system genes in BP and UP disorders.

The present study included 42 unrelated patients with BP disorder, type I (BPI; 25 females and 17 males; age 31–70 years; mean age at onset; 31.7 ± 5.7 years). Forty-one unrelated patients were suffering from UP recurrent major depression (UPR; 33 females and 8 males; age 30–74 years; mean age at onset, 39.6 ± 10.6 years). All were recruited among in- or outpatients of the "Rebro" and "Vrapče" psychiatric hospitals in Zagreb, Croatia. Patients had previously been assessed for lifetime psychopathology by trained clinicians, using clinical interviews and hospital case notes. At the time of the study, all patients were interviewed by the Schedule for Affective Disorders and Schizophrenia, Lifetime Version (SADS-L) [Endicott and Spitzer, 1978], by one independent experienced psychiatrist (L.O.), and only those who fulfilled Research Diagnostic Criteria (RDC) for BPI disorder and UPR major depression were included. In our sample, 16 BPI and 10

*Correspondence to: Prof. Dr. C. Van Broeckhoven, Ph.D., Neurogenetics Laboratory, Department of Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium.

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UPR patients were positive for family history as defined by at least one first-degree relative suffering from major affective disorders. The information on family members was collected from patients, but was also confirmed by reliable family members and medical records. The same diagnostic procedure was applied to select 71 age- and sex-matched unrelated controls (C; 53 females and 18 males) among the hospital staff and their friends. Only individuals with no personal or family psychiatric history were included. All patients and controls were from Croatian ancestry up to at least the second generation.

Genomic DNA was isolated from heparinized blood, using a standard phenol-chloroform extraction method [Maniatis et al., 1982]. The TH tetranucleotide repeat polymorphism [Polymeropoulos et al., 1991] was detected by [γ-^{32}P]-ATP end-labeling of one primer, followed by a standard polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis. The VNTR polymorphism in DRD4 [Nanko et al., 1993] was visualized using a fluorescence labeling protocol and was subsequently analyzed on an ABI-377 automated sequencer (Applied Biosystem, Foster City, CA). All genotypes were interpreted independently by two trained observers who were unaware of the patient diagnosis.

Exact tests for detecting deviations from Hardy-Weinberg equilibrium were performed using the Genepop version 2.0 software package [Raymond and Rousset, 1995]. The statistical significance of differences in specific allele and genotype frequencies, as well as homozygote/heterozygote frequencies between patient and control populations, was calculated using Fisher's exact test (two-sided tests).

The relative frequency, the observed number of alleles, and the number of homo- and heterozygotes for the BPI, UPR, and control groups are shown in Tables I (DRD4 polymorphism) and II (TH polymorphism). Results of Fisher's exact test for specific allele and homozygote/heterozygote frequencies are also given in these tables. No significant differences were observed at the single-allele level when comparing the UPR, BPI, or combined patient populations vs. controls. Our results on DRD4 are in agreement with other linkage and association findings of BP and the DRD4 gene locus [Sidenberg et al., 1994; De bruyn et al., 1994; Kennedy et al., 1993; Lim et al., 1994; Nanko et al., 1994; Perez de Castro et al., 1994]. To date there is no evidence for the involvement of the DRD4 gene in the pathogenesis of BPI disorder and UPR major depression.

For the TH polymorphism, the "1,2" genotype was more frequently observed among controls than in the patient group ($P = 0.04$; details not shown). However, this P value does not remain significant after applying a Bonferroni correction for multiple testing. When considering only BPI or UPR patients who were positive for family history, no significant differences were observed, either (details not shown). Some association studies have suggested that the TH gene may contribute to susceptibility to BPI and UPR major depression [Leboyer et al., 1990; Meloni et al., 1995; Kennedy et al., 1993; Verga et al., 1993]. However, in agreement with our results, others were not able to confirm these findings [Todd and O'Malley, 1989; Nöthen et al., 1990; Gill et al., 1991; Inayama et al., 1993; Körner et al., 1990, 1994; Kawada et al., 1995; Todd et al., 1996]. Intriguingly, in TH, the frequency of homozygotes in the BPI group was lower as compared to the control group (Fisher's exact test, $P = 0.08$, not significant). Within the BPI group, the number of homozygotes was also lower than expected under Hardy-Weinberg equilibrium (4 homozygotes observed; 8.8 homozygotes expected; $P = 0.017$, SE = 0.0005). A biological reason for the observed homozygote deficiency in the BPI group is unclear.

Failure to detect a significant association in this study suggests that the polymorphisms investigated play no major role in the etiology of the disease. However, the results may be influenced by many factors. First, it should be noted that the power of our study to detect odds ratios between 2.4–4.2, as observed for TH in other studies [Leboyer et al., 1990; Meloni et al., 1995], is 70–84%, 72–85%, and 82–90% for the BPI, UPR, and combined patient samples, respectively. As pointed out by Todd et al. [1996], true population differences or unnoticed population stratification might explain the conflicting results for the different association studies with TH. Furthermore, controls still within the age risk for developing mood disorders are also likely to diminish the chances of detecting potential associations.

TABLE I. Observed Allele Distribution of the DRD4 Polymorphism and Results of Fisher's Exact Test

	Relative frequency (number observed)				Fisher's exact test, P value		
Allele (bp)	BPI	UPR	BPI + UPR	C	BPI/C	UPR/C	(BPI + UPR)/C
2 (218)	0.09 (7)	0.15 (13)	0.12 (20)	0.08 (11)	1.00	0.18	0.34
3 (266)	0.04 (3)	0.06 (5)	0.05 (8)	0.03 (4)	1.00	0.49[a]	0.56
4 (314)	0.68 (53)	0.58 (50)	0.63 (103)	0.68 (88)	1.00	0.19	0.39
5 (362)	0.01 (1)	0.01 (1)	0.01 (2)	0.02 (3)	1.00	1.00	0.66
6 (410)	0.01 (1)	0.00 (0)	0.005 (1)	0.00 (0)	0.74	1.00	1.00
7 (458)	0.13 (10)	0.19 (16)	0.16 (26)	0.17 (21)	0.55	0.71	1.00
8 (506)	0.03 (2)	0.01 (1)	0.02 (3)	0.00 (0)	0.28	0.80	0.80
9 (554)	0.00 (0)	0.00 (0)	0.00 (0)	0.02 (3)	0.48	0.44	0.18
10 (602)	0.01 (1)	0.00 (0)	0.005 (1)	0.00 (0)	0.74	1.00	1.00
Total	1.00 (78)	1.00 (86)	1.00 (164)	1.00 (130)	0.38	0.26	0.27
Homozygotes	0.54 (21)	0.40 (17)	0.46 (38)	0.52 (34)	1.00	0.24	0.51
Heterozygotes	0.46 (18)	0.60 (26)	0.54 (44)	0.48 (31)			

[a]Largest odds ratio (95% confidence limits) observed, 1.944 (0.507, 7.456).

TABLE II. Observed Allele Distribution of the TH Polymorphism and Results of Fisher's Exact Test

	Relative frequency (number observed)				Fisher's exact test, *P* value		
Allele (bp)	BPI	UPR	BPI + UPR	C	BPI/C	UPR/C	(BPI + UPR)/C
1 (260)	0.29 (24)	0.32 (28)	0.30 (52)	0.31 (43)	0.76	0.88	1.00
2 (256)	0.19 (16)	0.17 (15)	0.18 (31)	0.24 (33)	0.50	0.32	0.26
3 (252)	0.13 (11)	0.11 (10)	0.12 (21)	0.09 (13)	0.38	0.66	0.47
4 (248)	0.14 (12)	0.09 (8)	0.12 (20)	0.10 (14)	0.39[a]	1.00	0.72
5 (244)	0.25 (21)	0.31 (27)	0.28 (48)	0.26 (37)	0.88	0.54	0.80
Total	1.00 (84)	1.00 (88)	1.00 (172)	1.00 (140)	0.70	0.79	0.72
Homozygotes	0.10 (4)	0.34 (15)	0.22 (19)	0.24 (17)	0.08	0.91	0.85
Heterozygotes	0.90 (38)	0.66 (29)	0.78 (67)	0.76 (53)			

[a]Largest odds ratio (95% confidence limits) observed, 1.500 (0.658, 3.418).

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